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On 1 Dec. 2006

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Malinda Adafit

OIPE DEC 05 2006 PATENT & TRADEMARK OFFICE

PATENT
Attorney Docket No.: 020130-001420US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:	Confirmation No. 2975
VANDER HORN, Peter B.	Examiner: Jeffrey S. Lundgren
Application No.: 10/627,592	Technology Center/Art Unit: 1639
Filed: July 25, 2003	DECLARATION UNDER 37 C.F.R. § 1.131
For: METHODS OF MAKING HYBRID PROTEINS	
Customer No.: 20350	

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Peter B. Vander Horn hereby declare that:

1. I am the inventor of the subject matter claimed in the above-referenced patent application.

2. I conceived of and reduced to practice the claimed invention in the United States *prior to* November 2002. Attached Exhibits A and B provide evidence of the completion of the invention. The dates on the Exhibits and unrelated information have been redacted. All redacted dates are prior to November 2002.

3. The present invention relates, in part, to methods of generating hybrid proteins. In the methods of the invention, at least two parent protein sequences that have a common biological activity are aligned and positions at which the parent amino acid residues are different are identified (such a position is referred to herein as a "divergent site"). The codons

that encode the differing residues are compared and a nucleic acid sequence encoding a protein that is a hybrid of the parent proteins is derived. This nucleic acid sequence includes degeneracies at codons that encode divergent sites. Such a degenerate codon has at least one nucleotide position in the codon that is variable such that the codon can encode multiple parental amino acid residues at the divergent site, depending on which nucleotide is incorporated at that codon position during synthesis of a nucleic acid molecule. Libraries can thus be generated in which members are hybrids that have amino acid residues from one of the parents at some of the divergent sites and, independently, amino acid residues from a different parent at other divergent sites. The library is then screened and functional hybrid proteins are identified.

4. Prior to November 2002, I aligned a parent Pfu polymerase protein sequence and a parent Deep Vent® polymerase protein sequence and identified differences in the amino acid sequences. An *E. coli* codon usage table was used to compare the various codons that can encode the differing amino acids. I created a nuclei acid sequence that alternatively encoded differing parental amino acid residues at sites of variation in the protein sequences.

5. Oligonucleotides were designed for synthesis to assemble together to form a full length polymerase gene to make a library encoding hybrid polymerase proteins. A copy of a laboratory notebook page showing the sequences of the oligonucleotides, including the positions that can encode alternative amino acid residues at sites that differ in the parent protein sequences, is provided in Exhibit A. The oligonucleotides were synthesized and assembled by overlap extension.

6. Functional polymerase proteins encoded by members of the library were identified. An example of a PCR analysis using an exemplary hybrid polymerase protein that was isolated from the library is shown in the copy of a laboratory note book page provided as Exhibit B. Exhibit B shows a gel with the products of amplification reactions performed using a hybrid polymerase, designated "PhS1". The hybrid polymerase amplified template DNA targets of 4, 5, 9, and 13 kb in length. The gel was obtained prior to November 2002.

PATENT

7. In view of the foregoing, I respectfully submit that it has been unequivocally established that the claimed invention was conceived of and reduced to practice prior to November 2002.

I further declare that all statements made herein of my knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statement and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that any such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Signed: Peter B. Vander Horn Dated: 10/30/2006

Peter B. Vander Horn

Attachments: Exhibits A and B

Notebook No. ________

PROJECT ________ Continued From Page ________

A

Project code: RGT1000 Vendor: *Operon* Date: *10/18/00* Ordered by: *Yan*

#	Name	Oligo Sequence (5' to 3')	Length (nt)	Scale (µmol)	Purify by	Cost($)
1	HY1a-RI/Nde	GGAATTCCATATGATCCTGGATGYTGACTACATCACTGAAGA	42	0.20	desalt	$35.70
2	HY1b	GGATGYTGACTACATCACTGAAGAHGGCAAACCGRTTATCCGTHTSTTCAAAAAAGAGAACGGCRAATTTAAG	73	0.20	desalt	$62.05
3	HY2	AGAATCATCTYTCAGCAGAGCGTAAATGTATGGACGAAAGRTGCGATCATRCTCAAYCTTAAATTYGCCGTTCTCTTTTTTCAA	84	0.20	desalt	$71.40
4	HY3	ATTTACGCTCTGCTGARAGATGATTCTHAGATTCAKGAAGTTARAAAAATCACTGSTGAGCGCCATGGCAAGATTGTTCGTATC	84	0.20	desalt	$71.40
5	HY4	GATTGGTTTGCCCAGAAATTTCTTCYYTACCTTTTCCRCATCAAYGATACGAACAATCTTGCCATGGCGCTCA	73	0.20	desalt	$62.05
6	HY5	GAAGAAATTTTCTGGCCARACCAATCRHGGTGTGCARACTGTATTYTCCAACATCCACAAGATGTTCCCA	68	0.20	desalt	$57.80
7	HY6	AATGGAATATCGTATTCGAAGATGTCAAYAACTGCAGRATGTTCGCGAAYTTTHTCGCGAATARTCGGAACATCTTGTGGATGTTCGA	88	0.20	desalt	$74.80
8	HY7	TTGACATCTTCGAATACGATATTCCATTTGCAAAGCGTTACCTCATCGACAAAGGCCTGATACCAATGGAGGGCGAKGAAGAAC	84	0.20	desalt	$71.40
9	HY8a	CAAACTCTTCGCCTTCGTCATAGACGGTTTCTATATCGAACGCCAGGAKCTTGAGTTCTTCHTCGCCCTCCATTGGTATCA	81	0.20	desalt	$68.85
10	HY8b-BclI	ATAGCTGATCATTATAATTGGGCCTTTASCAAACTCTTCGCCTTCGTG	48	0.20	desalt	$40.80
11	HY9-BclI	ATAATGATCAGCTATGCAGATGAARAMGAAGCAAAGGTGATTACTTGGAAAAAHATAGATCTCCC	65	0.20	desalt	$55.25
12	HY10	TGAGAAAGCGCTTAATCATCTCGCGCTCGGAAGATACAACCTCAACGTATGGCAGATCTATKTTTTTCCAAGTAATCACC	80	0.20	desalt	$68.00
13	HY11	GCGCGACATGATTAAGCGCTTTCTCARARTTATCCGCGAGAAGGATCCGGACRTTATCRTTACTTATAACGGCGACTCTTTTGAC	85	0.20	desalt	$72.25
14	HY12	CAGTTTAATACCGAGTTTTTTCTGCGCGTTTCRCCAGATATGGGARGTCAAAAGAGTCGCCGTTATAAGTAAYGATAA	77	0.20	desalt	$65.45
15	HY13	CGCGCAGAAAAACTCGGTATTAAACTGHCTHTCGGCCGTCATGGTTCCGAGCCGAAGATGCAGCGTHTCGGCGATATGAC	80	0.20	desalt	$68.00
16	HY14	AATTACATGATACAGGTCGAAATGGATACGACCCTTAAYTTCTACAGCGGTCATATCGCCGAKACGCTGCATCTTCGG	78	0.20	desalt	$66.30
17	HY15	ATCCATTTCGACCTGTATCATGTAATTHSCCGTACTATTAACCTCCCGACTTACACTCTCGAGGCTGTATATGAAGCAATT	81	0.20	desalt	$68.85
18	HY16-AccI	GCAATCTCATCGGCGTATACCTTCTCCTTCGGCTTACCAAAAATTGCTTCATATACAGCCTCGAGAGTG	69	0.20	desalt	$58.65
19	HY17-AccI	GCCGAAGGAGAAGGTATACGCCSATGAGATTGCARAGGCGTGGGAAWCCGGTRAGRRCCTCGAGCGTGTTGCAAAATACTCCATG	85	0.20	desalt	$72.25
20	HY18	TTCCATTGGGARGAATTCTYTGCCGAGTTCATAAGTCRCCTTTGCATCTTCCATGGAGTATTTTGCAACACGCTCGAG	78	0.20	desalt	$66.30
21	HY19	GAACTCGGCARAGAATTCYTCCCAATGGAARYTCAGCTCTCTCGCCTGGTTGGCCAACCACTGTGGGATGTTTCTCGTT	79	0.20	desalt	$67.15
22	HY20	GTTGCGTTCGTACGCTTTGCGCAGGAGAWACCACTCTACGAGGTTACCGGTGGAAGAACGAGAAACATCCCACAGTGGTTGGC	83	0.20	desalt	$70.55
23	HY21	CTCCTGCGCAAAGCGTACGAACGCAACGAASTGGCTCCGAACAAGCCAKHCGAASRAGAGTATSAACGCCGTCTCCGCGAGTCTT	85	0.20	desalt	$72.25
24	HY22	TTCCCAGAGGCCCTTTTCTGGCTCTTTAACAWAGCCACCAGYGTAAGACTCGCGGAGACGGCGTTSATACTCT	73	0.20	desalt	$62.05
25	HY23	TAAAGAGCCAGAAAAGGGCCTCTGGGAARRCHTCGTGTHCCTCGATTTTCGCKCTCTGTATCCGTCT	67	0.20	desalt	$56.95
26	HY24-AflIII	CCGGAGACACGTTGTGGGTAATGATAATAGACGGATACAGAGHGCCAAAATCGAGG	56	0.20	desalt	$47.60
27	HY25-AflIII	CACAACGTGTCTCCGGATACTCTCAACCKCGAGGGCTGCARARAHTATGATRTTGCTCCGSAAGTAGGCCACAAGTTCTGCAAGGAC	87	0.20	desalt	$73.95
28	HY26	TCGAGCAGAYGCYYCAGGAGAGACGGAATAAAGCCCGGGAKGTCCTTGCAGAACTTGTGGCCTACTT	67	0.20	desalt	$56.95
29	HY27	ATTCCGTCTCTCCTCRRGCRTCTGCTCGAKGAACGCCAARAGATTAAGHSCAAAATGAAGGHGKCCHAGGATCCGATTGAAAA	83	0.20	desalt	$70.55
30	HY28	AAGAGTTTGCGACGAKTTTAATCGCTYTTTGCGATAGTCGAGCAKTHTTTTTTCAATCGGATCCTKGGMCKCCTTCATTTTG	83	0.20	desalt	$70.55
31	HY29	AGCGATTAAANTCCTCGCAAACTCTTWTTACGGCTATTATGGCTATGCAAAAGCAGGCTGGTACTGTAAGGAGTGTGCTG	80	0.20	desalt	$68.00
32	HY30	GCTCCTTCCRCACGARCTCGATGTATTYGCGACCCCAAGCAGTAACGGACTCAGCACACTCCTTACAGTACCAGCGTGC	79	0.20	desalt	$67.15
33	HY31	AATACATCGAGYTCGTGYGGAAGGAGCTCGAAGAAAAGTTTGGCTTTAAAGTTCTCTACATTGACACTGATGGTCTCTAT	80	0.20	desalt	$68.00
34	HY32-Xba	AAATTCTAGAGCCTTTTTTCTTAATTTCCTCAGRCTYASCACCCGGAATAGTCGCATAGAGACCATCAGTGTCAATGTAGA	80	0.20	desalt	$68.00
35	HY33-Xba	AGGCTCTAGAATTTGTGAAATACATTAACKCCAAGCTCCCGGGTCTCCTGGAGCTCGAATATGAAGGCTTTTATRWGCGCGGC	83	0.20	desalt	$70.55
36	HY34	TTTGCCTTCTTCATCAATCASCGCATATYTCTTCTTCGTAACGAAGAAGCCGCGCWYATAAAAGCCTTCATATTCG	76	0.20	desalt	$64.60
37	HY35	ATATGCCGSTGATTGATGAAGAAGGCAAARTTATTACTCGTGGTCTCGAGATTGTGCGCCGTGATTGGAGCGAAATTGCGAAAG	83	0.20	desalt	$70.55
38	HY36	TTCTTCAACGTYGCCGTGTTTGAGAATAGYCTCGAGAACTYTAGCTTGAGTTTCTTTCGCAATTTCCTCCAATCACGGCG	81	0.20	desalt	$68.85
39	HY37	CTATTCTCAAACACGGCRACGTTGAAGAAGCTGTGARAATTGTAAAACAAGTAAYCSAAAAGCTCKCTAAHTATGAAATTCCGCC	85	0.20	desalt	$72.25
40	HY38	CGCCTTATACTCATGCAGCGGGCGAGTAATCTGCTCATAAATCRCGAGCTTCTCTGGCGGAATTTCATARTTAGHGAGCTTTT	83	0.20	desalt	$70.55
41	HY39	TACTCGCCCGCTGCATGAGTATAAGGCGATTGGTCCCACGTGGCTGTTGCAAAGARACTGGCTGCTARAGGCGTGAAA	79	0.20	desalt	$67.15
42	HY40-SacII	CATCGCCGCGGAGTACAATGTAGCCAATTACCATACCCGGTYTAAYTTTCACGCCTYTAGCAGCCAGTYTCTTTGC	76	0.20	desalt	$64.60
43	HY9-16F.Bcl	ATAATGATCAGCTATGCAGATGAA	24	0.20	desalt	$20.40
44	HY9-16R.Acc	GCAATCTCATCGGCGTATACCTTCTCC	27	0.20	desalt	$22.95
45	HY17-14F.Acc	GCCGAAGGAGAAGGTATACGC	21	0.20	desalt	$17.85
46	HY17-14R.Afl	CCGGAGACACGTTGTGGGTAATG	23	0.20	desalt	$19.55
47	HY25-32F.Afl	CACAACGTGTCTCCGGATACTCTC	24	0.20	desalt	$20.40
48	HY25-32R.Xba	AAATTCTAGAGCCTTTTTTCTTAA	23	0.20	desalt	$19.55
49	HY33-40F.xba	AGGCTCTAGAATTTGTGAAATAC	23	0.20	desalt	$19.55
50	HY33-40R.Sac	CATCGCCGCGGAGTACAATGTAG	23	0.20	desalt	$19.55
51	HY41-44R.Nhe	GTCATGCTAGCACTAGTGGGTACC	24	0.20	desalt	$20.40

* The price was calculated based on the special quote.

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PROJECT Program — Varying Denaturing times Notebook No. 23 B

Continued From Page 23

7-30 PHS1 L PCR - [dNTP]

0.5 sec @ f2.5 UV at 100% 0.3 or 0.36 mM dNTP
50, 60, 70, 80, or 90 mM KCl in EXT +2 mM Mg++
4, 5, 9, 13 kb old primer stocks --
PCR in 2 min/cycle 22 cycles

PHS1 0.36 mM dNTP's

PFS PFS buffer

PHS1 0.3 mM dNTP's

80 mM KCl | 60 | 70 | 80 | 92 | 50 | 60 | 70 | 80 | 90 mM KCl

0.36 mM to 0.3 mM dNTP's are about the same

0.3 mM has slightly more smearing

probably due to slightly higher effective [free Mg++] (i.e. less chelation by dNTP)

previous mixes partially at Room temp.

2.67x PHS1/Mix

Multiple Program Rxn's

2.67x PHS1 Mix 33.75 µl

2.67x Template 33.75 µl

4x Primer Mixes 22.5

90 µl ⇒ split into 4

2.67x PHS1 Mix		
10X EXT	58 µl	
1 M KCl	29	⇒ 50 mM
50 mM MgCl2	23.2	⇒ 2 mM
25 mM dNTP's	6.96	⇒ 0.3
PHS1	2.47	= 2.8 u/ml
ddH2O	97.6	
	217.2 ⇒ 580 µl	

Greater than 20 µl left

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